Comparison Microbial Killing Efficacy between Sonodynamic Therapy and Photodynamic Therapy

by Suryani Dyah Astuti

Comparison Microbial Killing Efficacy between Sonodynamic Therapy and Photodynamic Therapy

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ABSTRACT

Biofilm is a way used by bacteria to survive from their environmental conditions by forming colony of bacteria. Specific characteristic in biofilm formation is the availability of matrix layer, known as extracellular polymer substance. Treatment using antibiotics may lead bacteria to be to resistant. Other treatments to reduce microbial, like biofilm, can be performed by using photodynamic therapy. Successful of this kind of therapy is induced by penetration of light and photosensitizer into target cells. The sonodynamic therapy offers greater penetrating capability into tissues. This research aimed to use sonodynamic therapy in reducing biofilm. Moreover, it compares also the killing efficacy of photodynamic therapy, sonodynamic therapy, and the combination of both therapeutic schemes (known as sono-photodynamic) to achieve higher microbial killing efficacy. Samples used are *Staphylococcus aureus* biofilm. Treatments were divided into 4 groups, i.e. group under ultrasound treatment with variation of 5 power levels, group of light treatment with exposure of 75s, group of combined ultrasound-light with variation of ultrasound power levels, and group of combined light-ultrasound with variation of ultrasound power levels. Results obtained for each treatment, expressed in % efficacy of log CFU/mL, showed that the treatment of photo-sonodynamic provides greater killing efficacy in comparison to either sonodynamic and sono-photodynamic. The photo-sonodynamic shows also greater efficacy to photodynamic. So combination of light-ultrasound (photo-sonodynamic) can effectively kill microbial biofilm. The combined therapy will provide even better efficacy using exogenous photosensitizer.
Keywords: Photodynamic therapy, cahaya, Biofilm, ultrasound, sonodynamic therapy, photo-sonodynamic therapy, sono-photodynamic therapy, efficacy.

1. INTRODUCTION

Staphylococcus aureus is bacterial pathogen that one of main caused is chronic infection[1,2] . Their mode of survival to environment's conditions is carried out by forming biofilm [3]. Biofilm is regarded as the phase where bacteria forming their community via the quorum sensing [4,5]. This community will produce layer of matrix called extracellular polymer substance [5,6] When it formed biofilm, bacteria will be and persistent in compared to previous their planctonic phase[7] . Many antibiotic treatments cannot reduce biofilm, even it might lead to be resistances. Antibiotic resistance is induced by the *β-lactamase* enzym and efflux pump. Alternative therapy is able to reduce without make it resistance is photodynamic therapy. [8,9]

Photodynamic therapy has been practicing in reducing microorganism[10], is kind of therapeutic modality using photosensitizer and light (in certain wavelength) to produce reactive oxygen species (ROS)[11]. Photosensitizer can be endogenously required in the body. One of endogenous photosensitizer is porphyrin. *Staphylococci* bacteria the most is uroporphyrin[12] (89%) dan coproporphyrin III (68.3 % - 74.6%).[13]Absorbance spectra of *Staphylococcus aureus* tend to be around blue wavelength. By activating endogenous photosensitizer in bacteria, interactions between light and photosensitizer material will lead to ROS production[14]. These ROS molecules is toxic in nature and can cause cells apoptosis and necrosis. Deeper penetration of light and photosensitizer into cells regarded as the successful factors to produce greater apoptosis and necrosis.[15] This deeper penetration is aimed to reduce the target cells in tissue. One of the alternative to achieve this condition is by utilizing ultrasound wave.

Ultrasound has been widely applicated on imaging and medical. Application of ultrasound on therapy is called by Sonodynamic therapy (SDT). [16,17] It offers advantages in more penetration into biological tissue. The interactions between

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ultrasound wave and tissue, which is mostly composed of liquid, may produce microbubbles through acoustic cavitation process. These microbubbles will bind injected drug and leads to sonoporation process, i.e. penetrating into target tissue through a sonochemical process that is identical to the one of photodynamic in producing ROS.[18] sonosensitizer and photosensitizer is similar. [19,20] Sonosensitizer can also be endogenously required on the body like porphyrin.[21] The aimed of this study is to investigate killing efficacy between photodynamic therapy and sonodynamic therapy and combine of two therapies.

2. METHODOLOGY

2.1 Bacterial Strain

Isolate Staphylococcus aureus (ATCC 29823) was purchased in Faculty of Veterinary (Airlangga University). Isolate was grown overnight in Tryptic soy agar media (Oxoid, UK). Isolate was taken for growing suspensi in Triptic soy broth TSB (Merk, Germay) and incubated for 24 hours in incubator 38°C until it required optical density in 600nm 0.5 or ~ 10^6 CFU/mL.

2.2 Biofilm Growth

Biofilm was grown in microplate 96-well. Suspensi bacteria was put 100μL dan 20μL 2% sucrose and placed in every well. Sample was placed in shaker for 4 hours in order to form biomass on wall of well. Sample was rinse using Phosphate Buffered Saline (PBS) pH 7.4 in three times. Sample was grown in incubator 38°C for 48 hours.

2.3 Apparatus Treatment

The schematic for ilumination treatment is shown in Figure 1. It comprises of light source and generator of ultrasound source. Light source used to excite photosensitizer in photodynamic mode is diode laser, with output wavelength range of 404-425 nm, with measured output power of 82.02±0.11 mW. Exposure time setting used during the measurement was 75 sec. The generator as source ultrasound wave used in the experiments was the ultrasonic SKT-820, with frequency of 1 MHz, and in pulsed mode. Five (5) variation of power settings available are 28.76 W, 36.98 W, 45.2 W, 53.42 W, 61.64 W. Actual powers emitted by generator were measured using calorimetric method[22].

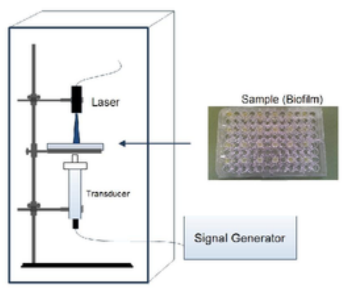

Fig 1. Schematic apparatus for ilumination treatment of sample.

2.4 Treatment

To determine the antimicrobial effect of treatments on *Staphylococcus aureus* biofilm, samples were distributed to 4 groups as follow: (1) Groups A sonodynamic (treated with ultrasound 5 min), (2) Groups B photodynamic (treated with laser 405 nm for 75 sec), (3) Groups C sono-photodynamic (treated ultrasound 5 min with 5 power variations followed with laser light exposure of 75 sec) (4) Group D photo-sonodynamic (treated laser light radiation for 75 sec and followed with ultrasound 5 min with 5 power variations). For each group the experiment was repeated at least 3 times. Treatments of samples done in complete dark room.

2.5 Crystal Violet Assay

Sample, had been treated, rinse by PBS three times and incubated for 24 hours. Sample that taken from incubator was rinse PBS three times. Sample was given 150μL 0.2% crystal violet and incubated for 15 minutes. Sample was rinse using dengan distilled steril water three times. Sample was given 50μL 33% glacial acetic acid (GAA) and measured using microplate reader S/N 17539 (Bio-rad, US) on 595nm.

2.6 Statistical analysis

Data was mean and standard error with repeatable n=4. Data is % efficacy dari log CFU/mL :

$$\%Efficacy = \left(\frac{\sum \text{Apoptosis/necrosis colony}}{\sum \text{no Treatment colony}} \right) \times 100\% \quad (1)$$

Data was statically analyzed using ANOVA one way test for difference each treatment. Difference of efficacy between PDT group and other treatment group was used independent sample t-test. Analysis test was used software SPSS ver19 for windows.

3. RESULT AND DISCUSSION

Result of sonodynamic was showed on Figure 2. Sonodynamic treatment was showed that exposure with 45.2 W produce 21.62%. Comparison each treatment was not different each other. It can be concluded that sonodynamic was not dependent on power. Comparison sonodynamic and photodynamic showed Figure 3. Result of data test showed that comparison photodynamic and sonodynamic was not significantly difference.

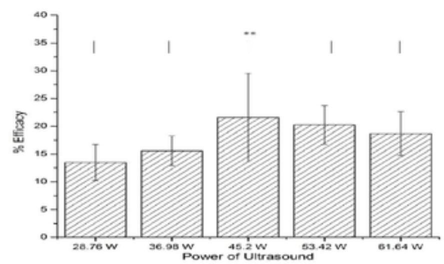

Figure 2. Result of Sonodynamic treatment. Symbol ** showed that significant level on ANOVA one way test p>0.05.

Figure.2 shows comparison of Sonodynamic treatment (with power variation) to Photodynamic (most left bar in the graph). Results data shows that photodynamic treatment (laser power of 82.02 mW and exposure time of 75 sec) does not show significant differences with sonodynamic treatment (with power variations and 5 minutes ultrasound exposure). Nevertheless, killing efficacy provides by photodynamic is 19.65% is a bit higher in comparison to sonodynamic at power of 28.76 W (13.462%), 36.98 W (15.576%), 61.64 W (18.685%), and a bit lower in comparison to sonodynamic at power of 45.2 W (21.618%), and for 53.42 W (20.23%).

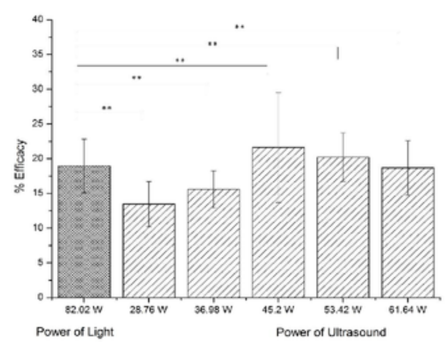

Figure 3. Efficacy comparison between sonodynamic (with power variations) and photodynamic (with endogenous sensitiser). Marker ** shows significant level of the independent test p>0.05.

Sono-photodynamic treatment refers to combined treatment of ultrasound followed by light treatment. This kind of treatment was started by sonodynamic treatments (with variations of power) and continued with photodynamic treatment with exposure time for 75 sec. Results of this kind combined treatments are shown in Figure. 4. There are two groups with difference of significant levels for every treatment. Comparison among sono-photodynamic with power variation of 28.76 W, 36.76 W, 53.42 W dan 61.64 W, they does not show differences, but not for the case with ultrasound power of 45.2 W. And Comparison among sono-photodynamic with power variation of 36.98 W, 45.2 W dan 53.42 W also does not show any significan differences. Sono-photodynamic treatment with endogenous sensitizer provides killing efficacy efficacy of 16.49%.

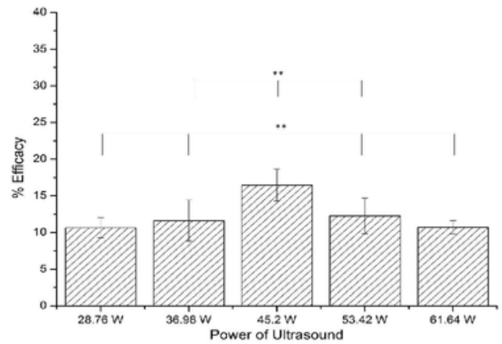

Figure 4. Results of Sono-Photodynamic treatment using endogeneous sensitizer with variations of ultrasound power Marker ** shows menunjukkan significant level in ANOVA one way test of $p>0.05$

Comparison between treatment of photodynamic vs sono-photodynamic is shown in Figure.5, and it shows significan differences except for ultrasound power of 45.4 W (16.488%). Photodynamic treatment with efficacy of 18.95% shows significant differences with sono-photodynamic treatments with ultrasound power of 28.75 W (10.664%), 36.76 W (11.638%), 53.42 W (12.26%) dan 61.64 W (10.72%). These results confirmed that photodynamic treatment provides greater killing efficacy in compare to sono-photodynamic one.

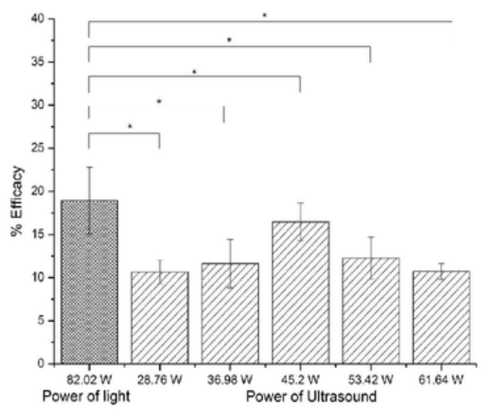

Figure 5. Result of comparison photodynamic and sono-photodynamic. Symbol * and ** showed significantly level on independent sample t test $p<0.05$ and $p>0.05$, respectively.

Photo-sonodynamic treatment refers to the one by firstly apply a light exposure and then followed by ultrasound exposure. Results of this kind of treatment are shown in Figure.6, which confirmed that all treatments of photo-sonodynamic does not show significant dependences to the power of ultrasound..

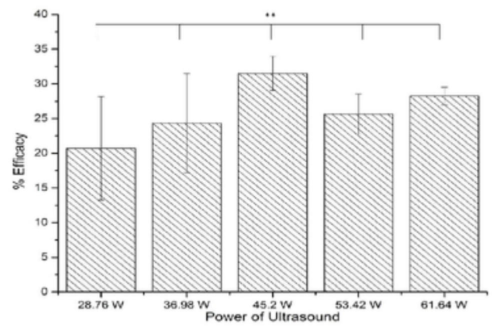

Figure 6. Results of Photo-Sonodynamic treatment (with variations of power) using endogeneous sensitizer Marker ** shows significant level in ANOVA one way test, i.e. $p>0.05$

Comparison photodynamic and photo-sonodynamic was showed in Figure 7. Comparison photodynamic (18.95%) and photo-sonodynamic power 45.2 W (31.526%), 53.42 W (25.65%) dan 61.64 W (28.251%) had significantly difference and had more efficacy than photodynamic.

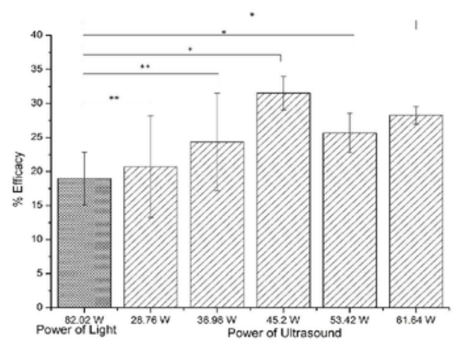

Figure 7. Perbandingan efficacy photodynamic dan photo-sonodynamic endogen dengan variasi daya ultrasound. Tanda * dan ** menunjukkan significant level pada independent test $p<0.05$ dan $p>0.05$, respectively.

Results from comparison between sonodynamic vs photodynamic does not show any significant differences ($p>0.05$). But numerically there is certain ultrasound power that will provide greater efficacy, at 42.5 W. Both photodynamic and sonodynamic have similarity, i.e. energy absorption will induces chemical process that produce free radicals [23,24]. Sono-photodynamic treatment will provide lower killing efficacy in comparison to photodynamic. The reason is not yet clear, and still need further investigations. It can be guessed that there are biological molecules that are activated by ultrasound or light that can prevent the formation of ROS, hence it will resistant with the combined treatment[25,26]. But the results from photo-sonodynamic treatment were reversed. Photo-sonodynamic showed to provide greatest killing efficacy to the *Staphylococcus aureus* biofilm.

From all group of samples treated using ultrasound, either sonodynamic, sono-photodynamic, or photo-sonodynamic, exposure using power of 45.2 W tend to provide greatest efficacy. It is possible that sonodynamic therapy require certain wave energy[27]. This process is started by the formation of microbubbles until reaching the collapses condition at sonoporation process due to inertia acoustic of the liquid. Microbubbles could catch molecules like molecules biologis or drugs for diffusing on membran channel[28]. It is provoked by mechanical effect from ultrasound. Moreover at the certain ultrasound energy, the induced sonochemical process will produce more ROSs. Under this certain energy absorption, it can be stated that the biological molecules (endogenous sensitizer) can be activated using ultrasound at 1 MHz frequency to produce microbubbles[29] and reduced the biofilm.

4. CONCLUSION

Comparison between photodynamic and sonodynamic treatments does not show significant difference in this research. Meanwhile it shows that photodynamic provides greater killing efficacy in comparison to sono-photodynamic but lower than photo-sonodynamic. In conclusion, therapy using ultrasound (sonodynamic) when combined with light exposure can be used as effective technique in reducing microbial biofilm. This kind of combined therapy will provide even better efficacy using exogenous photosensitizer.

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